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Genetic markers associated with divergent selection against the parasite *Marteilia cochillia* in common cockle (*Cerastoderma edule*) using transcriptomics and population genomics data

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The common cockle (Cerastoderma edule) plays an important role in marine ecosystems and represents a valuable socioeconomic resource for coastal communities. In 2012, the cockle beds from Ría de Arousa (Galicia, NW Spain) were seriously decimated by the protozoan Marteilia cochillia responsible for marteiliosis. We aimed to identify single nucleotide polymorphisms (SNP) markers potentially associated with resilience to marteiliosis to be used in marker-assisted selection programs for restoring affected cockle beds and recovering their production. For this, we carried out a population genomics approach using 2b-RADseq, where 38 naive samples (before the first detection of M. cochillia in 2012) from two beds of Ría de Arousa were compared with 39 affected samples collected in 2018/2019 (after several years of marteiliosis occurring in the area), collected either before (15 non-exposed samples) or during (24 exposed samples) the marteiliosis outbreak. Additionally, 767 differentially expressed genes (DEG) from a previous transcriptomic study addressed during the aforementioned 2018/19 marteiliosis outbreak, were evaluated to identify SNPs showing signals of selection. Using 2b-RADseq, 9,154 SNPs were genotyped and among them, 110 consistent outliers for divergent selection were identified. This set of SNPs was able to discriminate the samples according to their marteiliosis status (naive vs affected; exposed vs non-exposed), while another 123 SNPs were identified linked to DEGs associated with the level of infection across a temporal series. Finally, combining the population genomics and transcriptomics information, we selected the 60 most reliable SNPs associated with marteiliosis resilience. These SNPs were close to or within DEGs, and many of them were related to immune response (phagocytosis

and cell adhesion), defence, such as apoptosis, stress, and cellular cycle, among other functions. This set of SNPs will eventually be validated to develop a cost-effective genotyping tool for their application for obtaining cockle-resilient strains for marteiliosis.

KEYWORDS

SNP, bivalve, cockles, transcriptomics, population genomics, *Marteilia cochillia*, resilience

1 Introduction

The common cockle (*Cerastoderma edule*) is a marine bivalve species buried just below the surface of the sand or mud in intertidal and shallow subtidal areas of estuarine and marine coastal waters. It is widely distributed in the Northeast Atlantic, from the west coast of Africa in Senegal to the Barents Sea in Norway (Hayward and Ryland, 1995; Tyler-Walters, 2007). *C. edule* lives on average 2-4 years but can reach up to 10 years (Ponsero et al., 2009). It is a dioecious species that reaches sexual maturity at about one year of age (Maia et al., 2021). Like most bivalves, the common cockle releases gametes into the water, where external fertilization takes place (Moreira Sanmartín et al., 2016), and larvae remain in the water column for about 30 days (Creek, 1960), which allows their dispersal by marine currents (more than 100 km; Coscia et al., 2020). The spawning season runs from March to October, reaching the peak of activity between July and September when the water temperature is higher (~20°C; Maia et al., 2021).

Cockles are a highly valued shellfish species due to the range of ecosystem services that provide, e.g., the ability to reshape the seabed and alter the sediment properties (Ciutat et al., 2006; Neumeier et al., 2006; Andersen et al., 2010) depending on the substrate type (Rakotomalala et al., 2015; Eriksson et al., 2017). Like other bivalves, cockles contribute to improve water quality as filter feeders (Carmichael et al., 2012; van der Schatte Olivier et al., 2020) and aid to maintain biodiversity either indirectly, increasing the production of microphytobenthos (Swanberg, 1991) or as a direct source of food for different species (Sutherland, 1982; Beukema and Dekker, 2005). Furthermore, cockles are largely appreciated as a feeding resource for humans; Europe reported captures of 24,237 tonnes of *C. edule* in 2019, with Denmark, UK, Spain, and Portugal being the main producers (Food and Agriculture Organization of the United Nations [FAO], 2021). However, inter-annual production is unstable depending on different biotic and abiotic factors such as bacterial, viral, and parasitic infections (Lauckner, 1983; Bower et al., 1994), predation (Sutherland, 1982; Mascaró and Seed, 2000; Beukema and Dekker, 2005), food limitation (Bos et al., 2006), over-exploitation (Ferns et al., 2000), and environmental changes such as water temperature, salinity, pollution and in recent years climate change (Møhlenberg and Kiørboe, 1983; Ducrotoy et al., 1991; Beukema and Dekker, 2005; Parada and Molares, 2008; Burdon et al., 2014; Rowley et al., 2014).

Galicia (NW Spain) holds the most productive shellfisheries in Spain (Subsecretaría Subdirección General de Análisis Coordinacion y Estadística, 2020). In 2012, massive cockle losses were recorded in the Ría de Arousa, which houses one of the most productive shellfisheries on the Galician coast, associated with the protozoan parasite *Marteilia cochillia* (Villalba et al., 2014). In the following years, marteiliosis outbreaks spread to the southern Rías of Pontevedra and Vigo, almost depleting cockle production in the area. An annual pattern outbreak was recorded since the first 2012 outbreak; cases of infection are detected in newly recruited individuals in summer/early fall, followed by a progressive increase of prevalence and mortality until reaching almost 100% of cumulative mortality in the next months (Iglesias et al., 2023).

The parasite *M. cochillia* is a protistan parasite that colonizes the epithelium of the digestive gland of cockles destroying digestive diverticula and causing death due to starvation (Montaudouin et al., 2021). The complete life cycle of *M. cochillia* has not been yet disclosed. Darriba et al. (2020) observed parasitic forms (sporangia) being released through faeces into the environment. Intermediary hosts are hypothesized for its transmission, similarly to what is suspected to occur in the flat oyster (*Ostrea edulis*) infected by *M. refrigens*, where copepods of the genus *Paracartia*, i.e., *P. grani*, are involved (Audemard et al., 2002; Carrasco et al., 2015; Carballal et al., 2019). In this natural scenario, generating effective preventive measures against parasite infection is complex. Increasing resilience against *M. cochillia* through breeding programmes is an appealing approach to diminish the impact of the parasite in cockle beds, as has been demonstrated before in other bivalves (Ford and Haskin, 1987; Ragone Calvo et al., 2003; Kube et al., 2011; Proestou et al., 2016; Smits et al., 2020). This approach has been tested in natural environments, e.g. the breeding program for *Saccostrea glomerata* to obtain strains resistant to winter mortality and Qx disease caused by *Bonamia roughleyi* and *Marteilia sydneyi*, respectively (Nell et al., 2000), and in controlled conditions, such as the increased survival to ostreid herpesvirus 1 (OsHV-1) in *Crassostrea gigas* (up to 61.8%) after four generations of selection (Dégremont et al., 2015). On average, response to disease resistance selection in molluscs was higher than any other traits, such as growth (15% *vs* 10% per generation; see review of Hollenbeck and Johnston, 2018).

Genomic strategies are essential to understand the genetic basis of host-parasite interaction, for controlling marteiliosis and, eventually, for its application in breeding programs. For example, in *Crassostrea gigas* genomic prediction of OsHV-1 resistance was

more accurate (around 19%) than family-based prediction (Gutierrez et al., 2020). Genomic resources of common cockle have recently increased in the framework of the COCKLES Interreg (EAPA_458/2016) and the Scuba Cancers (ERC-2016-STG) projects, which ensured a robust genetic baseline for that purpose. A population genomics approach using 2b-RADseq along with the chromosome-level genome assembly of the species (Bruzos et al., 2022) was applied to disentangle the demographic and environmental factors underlying the common cockle structure in the Northeast Atlantic (Vera et al., 2022). Furthermore, RNAseq was applied to identify differentially expressed genes (DEG) in the digestive gland across the different infection stages. In this study, 767 DEGs, among the ~ 9000 annotated in the cockle's transcriptome, were identified when comparing samples of different infection levels across the outbreak 2018/19, many of which related to key immune pathways (Pardo et al., 2022).

The main goal of our study was to identify SNPs (Single Nucleotide Polymorphism), associated with genomic regions related to marteiliosis resilience in common cockle from Ría de Arousa for their eventual application in breeding programs and management of cockle beds. For this purpose, we followed two complementary approaches: i) identification of SNPs associated with divergent selection using groups of samples subjected to differential parasite pressure, and ii) detection of SNPs linked to the DEGs detected in response to *M. cochillia* outbreak by Pardo et al. (2022) showing significant genetic differentiation across groups with different level of infection. We used 2b-RAD and RNAseq data for genotyping anonymous and gene-linked SNPs, respectively, and further, we explored their involvement in the immune response that could explain the resilience to the parasite. A set of the most consistent SNPs were included thinking on its future validation for their potential application in breeding programs and management of common cockle beds.

2 Materials and methods

2.1 Population genomics approach

2.1.1 Sampling and DNA extraction

The sampling sites analysed in this study were selected according to relevant information on cockle marteiliosis epidemics. Marteiliosis was first detected in cockles from Galicia (NW Spain), namely from Ría de Arousa, in 2012 (Villalba et al., 2014); since then, outbreaks were recorded in this ría starting every summer/early fall, affecting each newly recruited annual cohort, and causing mass mortality (Iglesias et al., 2023). According to this information and the goals of the study, a total of 79 individuals were collected from two shellfish beds of Ría de Arousa (Table 1; Figure 1): i) 40 individuals in January 2012 before the first detection of *M. cochillia* (naive samples: NS) from Lombos do Ulla (SLO12) and O Sarrido (SSA12); and ii) 39 individuals from Lombos do Ulla in the 2018/19 period, after several generations of marteiliosis pressure ("affected"- samples, AS); among these, 15 juveniles were collected in spring 2018, before the annual marteiliosis outbreak (SLO18; non-exposed samples: NES), and 24 cockles mostly from a single cohort in spring 2019 (only four samples from September 2018) during the 2018/19 marteiliosis outbreak (SLO19; exposed samples: ES). A small portion of the gills was extracted from each individual and stored in 100% ethanol at 4°C. Genomic DNA was extracted from gills using the e.Z.N.A. E-96 mollusc DNA kit (OMEGA Bio-tech), following the manufacturer's recommendations. The quality and quantity of DNA were assessed with NanoDrop® ND-1000 (Nanodrop Technologies).

2.1.2 SNP calling and genotyping

Genotyping was performed following the 2b-RAD genotyping-by-sequencing (GBS) protocol (Wang et al., 2012). In brief, we obtained millions of 36 bp fragments in each sample produced by the digestion of genomic DNA with the A*lfI* IIb restriction enzyme (RE) (Thermo Fisher), which cut DNA at both sides of the RE site. 2b-RAD libraries were constructed at the Genomics Platform of Universidad de Santiago de Compostela (USC) and delivered to the FISABIO Platform (Valencia, Spain) for sequencing in a NextSeq 500 sequencer (Illumina). Then, reads from each individual were aligned to the common cockle genome (Bruzos et al., 2022) using Bowtie 1.1.2 (Langmead et al., 2009), and SNP calling was performed with Stacks 2.0 (Catchen et al., 2013; Rochette et al., 2019), following the parameters described by Vera et al. (2022). The RAD-tag SNP panel reported by Vera et al. (2022) mapped in the common cockle genome (Bruzos et al., 2022) was used as reference for genotyping to make feasible comparison with previous studies. Finally, some SNPs/RAD-tags or individuals were removed from the data using Plink 1.9 (Purcell et al., 2007) according to the following criteria: i) SNPs deviated from Hardy-Weinberg proportions ($p < 0.05$) in at least two sampling sites; ii) RAD-tags with more than 3 SNPs; iii) SNPs with missing data in > 50%

TABLE 1 *Cerastoderma edule* samples analysed from Ría de Arousa for the population genomics approach.

Sampling site	Date	Code	N	1st detection status	2018/19 outbreak status
Lombos do Ulla	Jan 2012	SLO12	20	NS	NES
O Sarrido	Jan 2012	SSA12	20*	NS	NES
Lombos do Ulla	May 2018	SLO18	15	AS	NES
Lombos do Ulla	April 2019	SLO19	24	AS	ES

NS and AS: naive (samples collected before 1st marteiliosis detection) and affected (samples collected after six years of marteiliosis outbreaks), respectively; NES and ES: non-exposed (samples collected before detection of the 2018/19 marteiliosis outbreak) and exposed (samples collected during the 2018/19 marteiliosis outbreak), respectively. *Two individuals were discarded from this sample after 2b-RAD filtering, totalling 18 individuals for analyses.

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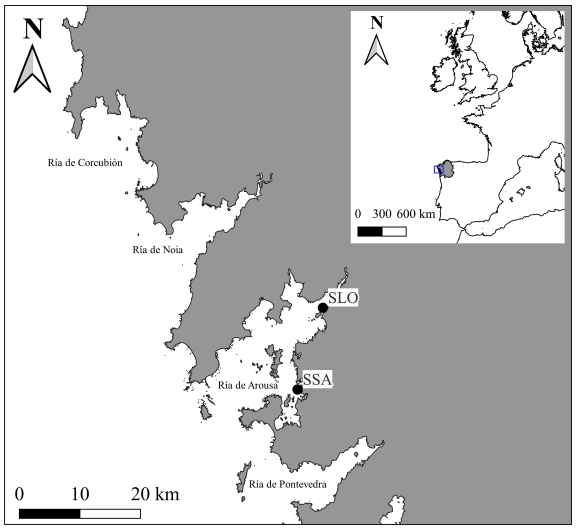

FIGURE 1
Study area showing the two sampled *Cerastoderma edule* beds in the Ría de Arousa (Galicia, NW Spain). Geographic Coordinate System – EPSG:25829 – SLO: Lombos do Ulla (518449 - 4719641); SSA: O Sarrido (514263 – 4706171).

individuals, and iv) individuals with < 30% of the SNP panel genotyped in any of the sampling sites.

2.1.3 Genetic diversity

The different subsets of SNPs used for analyses were extracted in Genepop format using the R package GENEPOPEDIT 1.0 package (Stanley et al., 2017). Allelic richness (Ar), observed (Ho) and expected (He) heterozygosity, and intrapopulation fixation index (F_{IS}) were calculated for each sample site to assess genetic diversity with the R package DiveRsity 1.9 (Keenan et al., 2013) (function "divBasic") with 1000 bootstraps. Departure from Hardy-Weinberg equilibrium (HWE) was estimated with exact tests using the enumeration method with GENEPOP 4.7.5 (Rousset, 2008).

2.1.4 Divergent selection for marteiliosis: Outlier detection

Two statistical approaches were performed to detect consistent outlier loci related to divergent selection against the neutral genomic background: i) the Bayesian F_{ST}-based method implemented in BAYESCAN v2.01 (Foll & Gaggiotti, 2008) was run using default parameters (i.e., 20 pilot runs; prior odds value of 10; burn-in of 50,000), 100,000 iterations and a sample size of 5,000; ii) the FDIST F_{ST} method implemented in ARLEQUIN v3.5 (Excoffier and Lischer, 2010) which uses a maximum likelihood approach (Beaumont & Nichols, 1996) was applied to incorporate *a priori* information regarding population structure with 100,000 simulations and 100 demes. For this purpose, we considered two scenarios where significant changes at specific genomic regions could hypothetically occur as a consequence of the differential selective marteiliosis pressure regarding the neutral background (Table 1): i) a temporal criterion (2012 *vs* 2018/19 outbreak), where naive samples (NS: SLO12, SSA12) constituted one group and affected samples (AS: SLO2018, SLO19) another; and ii) an exposure criterion, where samples from non-exposed cockles (NES: SLO12, SSA12 and SLO18) were grouped and compared to exposed samples (ES: SLO19). We used more strict parameters for ARLEQUIN (three technical replicates at $p < 0.01$), since this approach is more prone to false positives and a standard $p < 0.05$ for BAYESCAN, since it follows a more conservative approach (Narum and Hess, 2011).

All outliers detected were mapped on the *C. edule* genome (Bruzos et al., 2022), and those close to DEGs or another outlier (± 250 kb) were considered the most consistent ones (Population Genomic Candidates - PGCAND). Minor allele frequency (MAF) obtained with R package "adegenet" (Jombart, 2008) using "minorAllele" and "tab" functions respectively were calculated for further filtering steps.

2.1.5 Population structure

Global and pairwise relative coefficients of genetic differentiation (F_{ST}) were calculated between cockle sampling sites with GENEPOP 4.7.5 and R package StAMPP 1.6.2 with the 'stamppFst' function (Pembleton et al., 2013) using 10,000 bootstraps to calculate 95% confidence interval to test the null hypothesis ($F_{ST} = 0$). Genetic structure was additionally investigated through STRUCTURE 2.3.4 (Pritchard et al., 2000) using the R package ParallelStructure 1.0 (Besnier and Glover, 2013) to identify the most likely number of population units (K) in the samples. This program uses a Bayesian clustering approach to explore the population genetic units (clusters) using genotyping data. The program assigns the proportion of the genome that belongs to each of the clusters identified in each individual. Tests were performed without *a priori* information regarding the origin of samples, using an admixture model with correlated allele frequencies and burn-in of 100,000 iterations and 200,000 Markov Chain Monte Carlo steps. The number of K tested ranged from 1 to 5 (the number of sampling sites +1). For each K, ten replicates were performed to increase statistical confidence. The optimal number of K was estimated using the website program StructureSelector (Li and Liu, 2018) using different approaches: deltaK (Evanno et al., 2005), Mean LnP (K) (Pritchard et al., 2000) and those published by Puechmaille (2016). Graphical outputs were obtained with CLUMPAK (Kopelman et al., 2015). Further, a Discriminant Analysis Principal Component Analysis (DAPC) was performed with the R package adegenet to complement the STRUCTURE analysis. First, "find.cluster" function was used to assess the number of clusters in the population determining the optimal number of subpopulations with the Bayesian Information Criterion (BIC). Then, a cross-validation function was performed to detect the best number of principal components (PCs) given by the smallest mean square error (RMSE).

2.2 Transcriptomics approach

Pardo et al. (2022) identified 767 differentially expressed genes (DEG) in cockles collected before (July 2018) and at three different

times (November 2018, April 2019 and July 2019) during a natural outbreak of *M. cochillia* in 2018/2019 in Lombos do Ulla. Samples were classified histologically according to the level of infection as non-infected, mild, moderate and heavily infected. Then, DEGs were detected across a temporal series and according to the level of infection during the 2018/19 outbreak in Ría de Arousa. RNAseq data from these 767 differentially expressed genes (DEG) was used to call associated SNPs and estimate allele frequencies in each sample using SAMtools 1.9 (Li et al., 2009) with the following parameters: –skip-indels, –adjust-MQ 0, –max-depth 250. Then, SNPs were investigated for their association with the level of infection in exposed samples taken at three different times during a parasite outbreak (T1, T2 and T3). Samples were classified and pooled according to their level of infection across time using histopathology (Iglesias et al., 2023): I0: non-infected; I1: early infection; I2: moderate infection; I3: heavy infection; I4: final infection stage (Table 2). Allele frequency, missing data, expected heterozygosity and minimum allele frequency (MAF) were estimated per locus from the VCF file using all exposed samples (N = 50). SNPs that fitted the cut-off criteria of MAF > 0.05 and missing data < 30% were selected and mapped into the cockle's genome (Bruzos et al., 2022). Finally, the highest polymorphic SNP with the lowest missing data per DEG was chosen among those available.

Assuming the presence of genetic variation at DEGs related to marteiliosis response in Lombos do Ulla samples, we hypothesized that if divergent selection was occurring due to selective pressure, associated SNPs would show genetic differentiation between samples according to their level of infection, to say, on average exposed but non-infected samples would carry allelic variants related to resilience at a higher frequency, while heavily infected ones, would do for susceptibility variants. To increase statistical power, exposed samples were grouped into three sets according to their infection level: i) non-infected (15 individuals); ii) early/moderately infected (22 individuals), and iii) heavily infected/final stage of infection (13 individuals). Global F_{ST} was estimated for all selected SNPs using those three groups and their significance was estimated with exact tests ($p < 0.05$) using Genepop 4.7.5. When possible, the two most polymorphic SNPs were retrieved per DEG. Candidate SNPs were finally selected from the transcriptome approach (TCAND) from those showing the highest genetic differentiation ($p < 0.05$).

TABLE 2 *Cerastoderma edule* samples collected in Lombos do Ulla used for the transcriptomics approach classified by infection level.

Date	Code	I0	I1	I2	I3	I4
Nov-18	T1	5	5	5	5	4
Apr-19	T2	5	5	5	3	1
Jul-19	T3	5	0	2	0	0

I0: non-infected; I1: early infection; I2: moderate infection; I3: heavy infection; I4: final infection stage.

2.3 Final selection SNP panel

Once candidates from the population genomics approach (PGCAND) and transcriptomics (TCAND) were identified, a final set of SNPs was selected to design a cost-effective molecular tool including ~60 SNPs to be eventually used for obtaining resilient strains to marteiliosis. Significant SNPs from both approaches were first filtered by missing data < 30% and MAF > 0.05 and then by technical issues related to primer design and multiplexing. This preselected SNP set was next sorted according to the statistical confidence to be under divergent selection (*p*-value) and further prioritize for consistency using the following criteria: i) signals of selection in more than one approach (i.e., temporal and exposition among PGCAND); ii) more than one SNP detected in less than 250 kb; and iii) higher genetic diversity and lower missing data. Finally, when more than one outlier was found in the same gene or region (± 250 kb), only one of the markers was selected.

3 Results

3.1 Population genomics approach

Genetic diversity and structure were investigated with different sets of SNPs on several groups of samples related to the strategies followed to identify the most reliable set of outlier markers associated with divergent selection against marteiliosis (PGCAND outliers). Analyses were performed with i) the whole polymorphic SNP dataset; ii) the divergent outlier dataset; and iii) the neutral dataset, defined after removing outliers from the whole data.

3.1.1 Outlier detection and mapping

After filtering, 9,154 SNPs were retained (Table S1) from the SNP panel reported by Vera et al. (2022), and among them, 6,252 SNPs (68.3%) were polymorphic in our collection. The detection of outlier markers was performed using the 6,252 polymorphic SNPs, representing 1.6 SNPs/Mb according to the common cockle genome size (794 Mb; Bruzos et al., 2022), under the null hypothesis of neutrality across the whole genome. Thus, outliers with F_{ST} significantly above the neutral background were considered under divergent selection, while those with F_{ST} below the neutral background were considered under stabilizing selection. The two statistical methods implemented in BAYESCAN and ARLEQUIN programs, respectively, were applied and tested in the temporal (naive *vs* affected) and exposure (non-exposed *vs* exposed) scenarios (see Materials and Methods). BAYESCAN, the most conservative and sensitive to sampling error method, only detected one outlier under divergent selection in the temporal scenario, while ARLEQUIN detected a total of 213 consistent outliers ($p < 0.01$ in three technical replicates), 74 in the temporal (t) and 156 in the exposure (E) scenarios, respectively, 17 of them shared in both scenarios, including the one detected by BAYESCAN (Table S2). No outliers under stabilizing selection were detected with any of both methods. All the 213 consistent divergent outliers were mapped in the *C. edule* genome and additionally checked for their proximity to

other outliers (< 250 kb) (Table 3) or to any of the 767 DEGs reported by Pardo et al. (2022) (Table S3). A total of 110 SNPs (10 shared between both scenarios) met the criteria and were selected as the most reliable set of SNPs from the population genomics approach (PGCAND).

3.1.2 Genetic diversity

Genetic diversity using the whole 9,154 SNP dataset was slightly but significantly higher ($p < 0.05$) in samples from 2012 as compared to those from the 2018-19 period both for allelic richness (Ar: 1.362 *vs* 1.321) and expected heterozygosity (He: 0.085 *vs* 0.077). None of the samples showed global deviation from HWE ($p > 0.05$), although there was a significant deficit of heterozygotes in most samples using the confidence interval approach (Table 4A). Results were very similar when considering only the neutral SNPs (data not shown). However, when using the 110 PGCAND outlier panel (Table 4B), genetic diversity was lower in non-exposed *vs* exposed samples (NES *vs* ES: Ar: 1.330 *vs* 1.672, He: 0.100 *vs* 0.169, respectively; $p < 0.05$, Mann-Whitney tests) and further, a highly significant departure from HWE involving a remarkable heterozygote deficit was detected in the exposed sample (SLO19 F_{IS} = 0.364; $p < 0.001$). Interestingly, the high value detected in the exposed sample was mainly due to the 156 outliers of the exposure scenario (F_{IS} = 0.456; $p < 0.001$), while it remained significant but very similar across the four populations when the 74 outliers of the temporal scenario were compared (F_{IS} SLO12: 0.293; SSA12: 0.307; SLO18: 0.287 and SLO19: 0.290).

3.1.3 Genetic structure and differentiation

The global F_{ST} = 0.0032 showed low but significant ($p < 0.01$) genetic differentiation with the whole SNP dataset. Pairwise F_{ST} comparisons showed low and usually non-significant differentiation when using the neutral panel (6,006 SNPs), but highly significant differentiation when using the 110 most consistent outlier SNPs, especially when comparing naive (2012) and affected (2018/19) groups (average F_{ST} = 0.091), but also between non-exposed and exposed samples in the 2018/19 outbreak (F_{ST} = 0.033; Table 5).

The clustering method of STRUCTURE applied to the neutral dataset showed K = 1 as the optimal number of clusters according

TABLE 3 Genomic location of consistent SNPs detected in *Cerastoderma edule* following population genomics and transcriptomics approaches along with the differentially expressed genes reported by Pardo et al. (2022).

Chromosome	Size (bp)	Total PGCAND outliers	PGCAND selected	DEGs	TCAND
1	64609245	14 (8E, 6t)	5 (3E, 2t)	58	13
2	56319168	16 (11E, 5t)	10 (7E,3t)	58	10
3	55987847	20 (15E, 5t)	11 (9E, 2t)	59	10
4	52087795	14 (7E, 4t, 3S)	3 (1E, 2S)	44	4
5	50828891	21 (12E, 7t, 2S)	11 (6E, 5t)	55	8
6	40237005	9 (5E, 4t)	5 (2E, 3t)	47	9
7	39934596	7 (5E, 2t)	3 (3E)	40	8
8	39684391	11 (5E, 6t)	7 (3E, 4t)	41	6
9	39070162	4 (4E)	2 (2E)	37	9
10	38264924	15 (8E, 5t, 2S)	11 (5E,5t, 1S)	54	13
11	38197540	5 (4E, 1S)	2 (1E, 1S),	35	2
12	36327582	10 (6E, 1t, 3S)	6 (4E, 2S)	35	2
13	35955507	10 (9E, 1S)	5 (4E, 1S)	36	3
14	33816358	10 (8E, 2t)	5 (4E, 1t)	33	5
15	31726440	15 (9E, 3t, 3S)	6 (3E, 1t, 2S)	32	4
16	31510408	7 (5E, 1t, 1S)	3 (3E)	29	5
17	26587828	8 (6E, 2t)	6 (4E, 2t)	34	6
18	22603465	8 (6E, 1t, 1S)	6 (4E, 1t, 1S)	20	4
19	21711631	5 (3E, 2t)	3 (2E, 1t)	19	2
Minor scaffolds		4 (3E, 1t)	0	1	0
Total	755,460,783	213 (139E, 57t, 17S)	110 (70E, 30t, 10S)	767	123

Chromosome size according to Bruzos et al. (2022); DEGs, differentially expressed genes; PGCAND, candidates from population genetics approach: E (exposure), t (temporal) and S (shared in t and E); TCAND: candidates from transcriptomics approach.

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TABLE 4 Genetic diversity in *Cerastoderma edule* samples from Ría de Arousa with: A) Whole dataset (9154 SNPs); B)110 PGCAND dataset.

A)						
		Complete SNP dataset				
Code	N	Ar	Ho	He	F_{IS}	CI
SLO12	20	1.367	0.080	0.085	**0.048**	0.021 – 0.068
SSA12	18	1.357	0.079	0.085	**0.073**	0.029 – 0.111
SLO18	15	1.281	0.072	0.073	0.013	-0.055 – 0.062
SLO19	24	1.361	0.075	0.081	**0.070**	0.050 – 0.084
B)						
		110 PGCAND				
Code	N	Ar	Ho	Code	N	Ar
SLO12	20	1.372	0.091	0.107	**0.149**	0.075 – 0.215
SSA12	18	1.369	0.107	0.120	0.113	-0.011 – 0.232
SLO18	15	1.248	0.061	0.074	**0.176**	0.022 – 0.288
SLO19	24	1.672	0.107	0.169	**0.364**	0.280 – 0.449

N, number of individuals for each location; Ar, allelic richness; Ho, observed heterozygosity; He, expected heterozygosity; F_{IS}, intrapopulation fixation index. CI, Confidence interval 95%.
In bold type significant values ($p < 0.05$).
Sample codes are shown in Table 1.

to all K estimators described by (Puechmaille, 2016) and K = 2 with ΔK and LnP (K) (Figure 2). Nevertheless, the second population unit would be spurious according to the criterion defined by Puechmaille (2016). Results with 110 PGCAND yielded different optimal Ks depending on the estimator used. According to LnP (K) (Pritchard et al., 2000), seven distinct groups were detected, while Puechamaille's estimators and ΔK reported optimal K = 3 differentiating naive (NS: SLO12/SSA12), non-exposed (NES: SL18) and exposed (ES: SLO19) samples. DAPC analyses yielded the lowest Bayesian information criterion for K = 1 with the neutral panel, and K = 2 (BIC K = 2; 161.754) with the 110 PGCAND, but K = 3 (162.722) rendered a slightly lower value showing a sample differentiation similar to that by STRUCTURE (Figure 3). All in all, two or three groups were identified, respectively, in the exposure (2 groups) and temporal (3 groups) scenarios, the non-exposed group (SLO18) being in-between the two more differentiated naive (SLO12, SSA12) and the exposed (SLO19) groups (Figure 3B).

3.2 Transcriptomics approach

More than five million SNPs were initially identified from the RNAseq reads aligned against the common cockle reference genome, which were reduced to > 950,000 high-quality SNPs after filtering with SAMtools. Among them, ~ 45,000 SNPs were inside the 767 DEGs reported by Pardo et al. (2022). After filtered by MAF (> 0.05) and missing data (< 30%), a total of 12,753 SNPs were obtained. Two SNPs with the highest MAF and the lowest missing data were retained per DEG (when available), thus constituting a total of 1,418 SNPs more manageable dataset (Tables S3, S4). Among the three groups of samples classified according to the level of infection (I0: no infection, I1: early/moderate, I3: heavy/final), 123 SNPs showed significant genetic differentiation ($p_{FST} < 0.05$) and constituted the set of candidate markers from the transcriptomics approach (TCAND) (Tables 5, S5). Among them, 41 SNPs showed a progressive increase (or decrease) in the frequency of the reference allele across the three

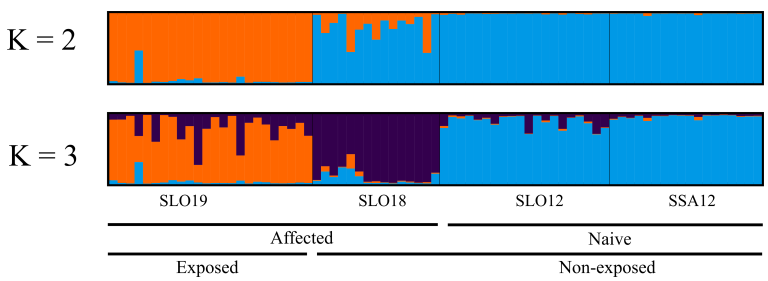

FIGURE 2
Population structure of *Cerastoderma edule* beds analysed with STRUCTURE with 110 PCAND dataset for K = 2 and K= 3). Each individual is represented by a vertical bar and its colour is proportional to the posterior probability assigned to each cluster.

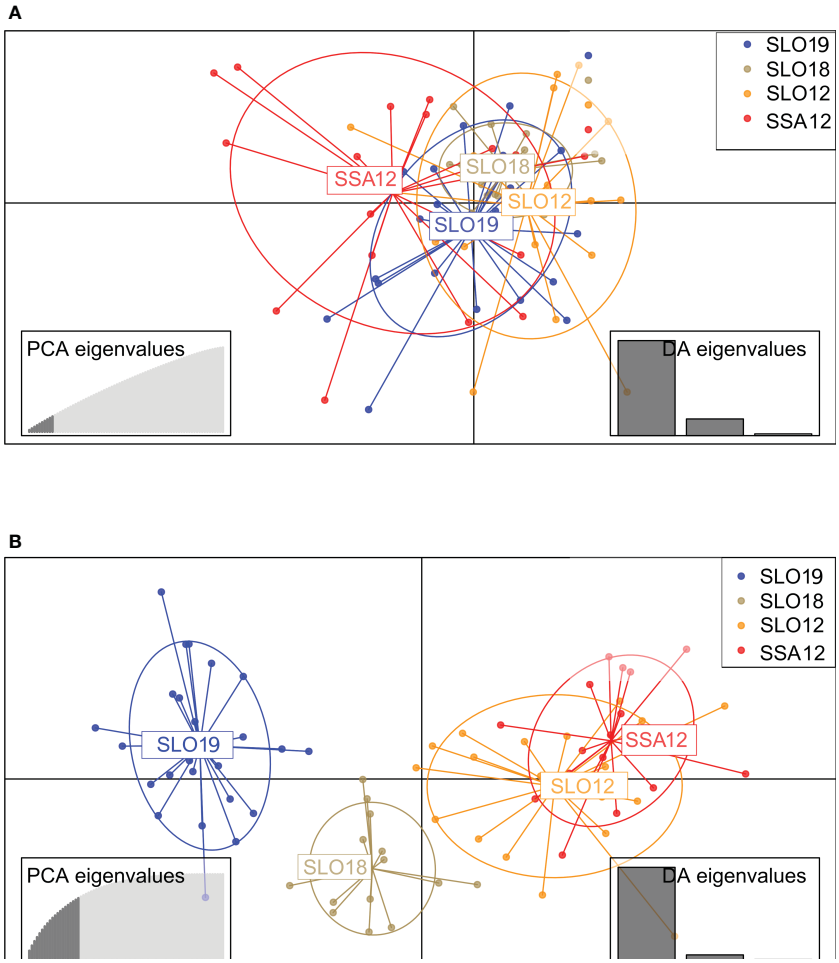

FIGURE 3
Discriminant Analysis of Principal Components (DAPC) plots of cockles *Cerastoderma edule* using: **(A)** neutral dataset (10 PCs; 18% of variance explained); **(B)** 110 PCAND dataset DAPC (20 PCs, 72% of explained variance). Codes are shown in Table 1. PCs retained according to the cross-validation method with the lowest RMSE are shown at the left bottom of each panel.

infection level groups (Table S5). Chromosomes (C) C1 and C10 housed the highest number of candidates (13 SNPs) (Table 3).

3.3 Final selection SNP panel

A final marker selection was made with the most suitable SNPs from the 110 PGCANDs and the 123 TCANDs to constitute a set of markers to be further validated by their technical and marteilioisis diagnosis usefulness (Table S6). PGCAND and TCAND were first preselected by MAF (> 0.05), missing data (< 30%) and technical criteria (± 100 bp flanking regions lacking additional polymorphisms), yielding a final set of 44 PGCAND and 38 TCAND. Then, they were ranked from the lowest to the highest genetic differentiation *p*-value for selection and additionally filtered to retain only one marker per genomic window (± 250 kb), and those with the highest F_{ST} and lowest missing data when more than one was available. A final panel of 60 SNPs was considered as a suitable set to define a cost-effective tool for validation (36 from PGCAND and 24 from TCAND approaches, respectively). These SNPs were placed in the cockle genome (Table 6). Chromosomes C5 and C10 housed the higher number of SNPs (5 and 8 SNPs respectively). Selected markers were related to catalytic functions and binding activities (17), such as the cathepsin family L, calmodulin, IgGFc-binding protein gene, Golgin subfamily A member, glutathione S-transferase sigma or proteasome related genes. Many of these genes have also been related to immune response (phagocytosis and cell adhesion), and defense, such as apoptosis, stress, and cellular cycle, among other functions (Table 6) (Niu et al., 2013; Nanut et al., 2014; Vigneron and Van den Eynde, 2014; Han et al., 2021).

4 Discussion

Cockle beds in Ría de Arousa experience annual outbreaks since 2012 (Villalba et al., 2014; Iglesias et al. 2023) due to the parasite *Marteilia cochillia*, which has collapsed its shellfishery. Although the parasite has only been recorded in restricted areas, namely Rías de Arousa, Pontevedra and Vigo in Galicia (Northwest Atlantic

TABLE 5 Pairwise F_{ST} matrix for *Cerastoderma edule* samples collected in 2012 (NS: naive) and in the 2018/19 period (AS: affected) in the Ría de Arousa, Galicia (NW Spain).

	SLO19	SLO18	SLO12	SSA12
SLO19	–	-0.001	-0.001	0.000
SLO18	**0.033**	–	0.001	**0.003**
SLO12	**0.080**	**0.065**	–	**0.002**
SSA12	**0.103**	**0.115**	0.002	–

Above diagonal: 6,006 neutral SNP dataset; below diagonal: 110 Candidate SNPs from population genomics approach (PGCAND). Significant values in bold ($p < 0.05$).

coast of Spain), Fangar and Alfacs bay in Catalonia (Northeast Mediterranean coast of Spain); Huelva (Southwest Atlantic coast of Spain); and Ría de Aveiro and Formosa Ras (North and South of Portugal, respectively) (Carrasco et al., 2013; Navas et al., 2018; Montaudouin et al., 2021; Iglesias et al., 2023), its presence threatens cockle production regardless that larval dispersal and connectivity between beds (Vera et al., 2022) could be aiding to recover recruitment every year (Iglesias et al. 2023).

Prevalence of marteiliosis and mortality of cockles have decreased since 2017 in Lombos do Ulla (Iglesias et al., 2023) and furthermore, batches from the naive shellfish bed of Noia (Ría de Noia, just north of Ría de Arousa) transplanted into Lombos do Ulla in 2017 and 2018 experienced much higher marteiliosis prevalence and mortality than those recruited in Lombos do Ulla during the same season. These observations led to hypothesize that Lombos do Ulla cockles had acquired certain resiliency to the parasite due to natural selection (Iglesias et al., 2019) and suggested that candidate genes and associated markers were probably underlying marteiliosis resilience. Thus, searching for those genetic markers was considered a main goal for recovering cockle production and preventing its expansion to other areas through breeding programs or appropriate shellfishery management. Similar approaches have been tackled for the identification of molecular markers for disease resilience in other mollusc species (de la Ballina et al., 2018; Ronza et al., 2018; Farhat et al., 2020; Leprêtre et al., 2021).

The immune system of molluscs lacks adaptative immunity and depends mostly on innate immune response, constituted by cellular and humoral responses (see review Allam and Raftos, 2015). Pardo et al. (2022) reported a set of DEGs associated with innate immune function, such as signal transduction, response to stimulus, cytoskeletal organization, pathogen recognition receptor (PRR), serine protease inhibition and antimicrobial response when analysing the transcriptomic response of cockles collected from Ría de Arousa during the same marteiliosis outbreak analysed in our study. Understanding the genetic basis of differential immune response may help to identify mechanisms conferring resilience or susceptibility to a particular disease. Moreover, detection of genetic markers associated with those differences, either responsible or not, but in linkage disequilibrium with the responsible variants, would be decisive for obtaining cockle strains resilient to the parasite.

We hypothesized that *M. cochillia* decreased prevalence and mortality rates recorded since 2017 in the inner area of the Ría de Arousa (Iglesias et al., 2019; Iglesias et al., 2023), are associated with selection of specific immune gene variants, which could eventually be identified using population genomics and transcriptomic approaches. Accordingly, outlier loci for divergent selection might be identified against the neutral background if appropriate cockle samples related to different marteiliosis pressures were compared. On the other hand, SNPs within DEGs showing genetic differentiation associated with the level of infection would be another relevant source of information for detecting consistent candidate genes holding allelic variants associated with resilience to the parasite. A similar approach has been recently reported in flat oyster *O. edulis*, where a major QTL related to resilience to *Bonamia ostreae* was identified (Vera et al., 2019; Sambade et al., 2022). Other selection models, such as overdominance, that have been reported for specific immune genes (Penn, 2002; Kekäläinen et al., 2009), could be operating and therefore our results would only explain part of the increased resilience. Furthermore, epigenetic imprinting is increasingly being claimed to be involved in immune memory in molluscs (Pradeu and Du Pasquier, 2018; Yao et al., 2021) and have also been suggested for bonamiosis resilience in flat oyster (Sambade et al., 2022).

Accordingly, we compared, on one hand, DNA samples preserved since 2012, corresponding to cockles recruited before the parasite's first detection (naive) *vs* those from the 2018/2019 period (affected), when the bed had been affected by marteiliosis for six years, the last ones including non-exposed (2018) and exposed (2019) samples to the parasite; and, on the other hand, RNAseq data from DEGs among samples with different levels of infection from the same outbreak (Pardo et al., 2022). The recent publication of the common cockle's genome (Bruzos et al., 2022) enabled to integrate all that information to look for more consistent signals of selection associated with the response to the parasite.

Using a previously validated 2b-RAD panel by Vera et al. (2022), we observed that genetic diversity in Arousa samples (He: 0.073 to 0.085) was similar to that reported in other studies in northwest Europe (He = 0.077 – 0.088, Vera et al., 2022), although the number of polymorphic loci was lower (6,252 in Ría de Arousa), an expected outcome considering the small geographic area studied. We detected a lower genetic diversity in the affected samples (SLO18/SLO19) than the non-affected ones from 2012, also expected considering the heavy mortalities and strong bottlenecks affecting cockle beds after consecutive marteiliosis outbreaks in the Ría de Arousa (average He: 0.077 *vs* 0.085; $p < 0.05$). Besides, we detected a slight heterozygote deficit ($F_{IS} > 0$) in all the samples with the whole and neutral SNP datasets, a usual observation in molluscs due to the presence of null alleles (see review Hollenbeck and Johnston, 2018). However, the heterozygote deficit was higher with

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TABLE 6 List of the 60 SNPs selected from transcriptomic (TCAND) and population genomics (PGCAND) approaches positioned in the cockle *Cerastoderma edule* genome selected for resilience to *Marteilia cochillia*.

SNP_ID	Chr	Position	Ref	Alt	F_{ST}	*p*-value	MD	MAF	Inside/closest gene – distance to gene
1_18985767	1	18,985,767	T	C	0.078	0.031	0.120	0.409	Cyclic nucleotide-binding domain-containing protein 2-like – Inside
152630_16	1	21,765,580	A	G	0.106	0.003	0.247	0.069	Calmodulin - ~120 kb
1_25594873	1	25,594,873	A	C	0.121	0.006	0.000	0.490	Kazal-like domain-containing protein – Inside
172379_8	2	7,331,175	G	T	0.086	0.010	0.065	0.096	BRISC and BRCA1-A complex member 1 – Inside
2_31820629	2	31,820,629	G	A	0.157	0.001	0.000	0.470	Uncharacterized protein LOC111119482 – Inside
270375_0	2	32,784,443	C	T	0.086	0.006	0.169	0.063	Centrosome and spindle pole associated protein 1-like ~57 kb
2_44323669	2	44,323,669	A	G	0.136	0.003	0.220	0.321	Thimet oligopeptidase - Inside
209292_33	3	10,407,569	T	C	0.109	0.004	0.013	0.227	3-phosphoadenosine-5-phosphosulfate synthase ~21 kb
3_12504155	3	12,504,155	A	C	0.131	0.006	0.000	0.420	Lysosomal alpha-glucosidase-like - Inside
3_26188137	3	26,188,137	C	T	0.081	0.019	0.000	0.420	Putative zyg-1-like serine/threonine protein kinase (Fragment) – Inside
3_33568023	3	33,568,023	G	A	0.089	0.014	0.000	0.290	RNA helicase – Inside
185992_6	3	52,822,008	G	T	0.096	0.003	0.234	0.057	Peroxisomal membrane protein PMP34 ~34 kb
221361_4	4	29,246,635	T	C	0.194	0.000	0.156	0.052	Guanine nucleotide exchange factor for Rab-3A-like ~3.5 kb
19806_17	4	30,868,560	A	G	0.106	0.003	0.299	0.411	Testis-expressed sequence 11 protein ~5.4 kb
4_45423046	4	45,423,046	T	C	0.092	0.014	0.000	0.380	Apolipoprotein D-like – Inside
182698_17	5	7,332,544	T	C	0.130	0.001	0.026	0.091	Low-quality protein: la-related protein 1B-like – Inside
39442_34	5	23,254,047	C	T	0.108	0.004	0.156	0.097	Kinesin-like protein KIF1C – Inside
5_29151780	5	29,151,780	T	A	0.097	0.006	0.000	0.210	Hexosyltransferase – Inside
166822_3	5	34,008,325	G	T	0.139	0.001	0.221	0.121	Folate_rec domain-containing protein – Inside
270079_29	5	35,790,787	T	C	0.172	0.000	0.273	0.155	FH2 domain-containing protein 1 ~28.7 kb
5_48857211	5	48,857,211	T	G	0.107	0.011	0.100	0.389	Cytochrome P450 4F22 – Inside
259542_9	6	8,587,257	C	T	0.108	0.004	0.156	0.077	Monocarboxylate transporter 12-like – Inside
206963_8	6	9,943,132	A	T	0.116	0.002	0.169	0.068	Gamma-secretase subunit PEN-2-like ~23.0 kb
6_11581379	6	11,581,379	T	A	0.178	0.000	0.000	0.420	26S proteasome non-ATPase regulatory subunit 9-like – Inside
6_32304661	6	32,304,661	C	A	0.279	0.000	0.300	0.143	NPHS1 adhesion molecule, nephrin – Inside
184559_32	7	2,881,173	C	T	0.098	0.005	0.247	0.092	Vacuolar protein sorting-associated protein 4B-like ~5.1 kb
7_20566394	7	20,566,394	T	A	0.088	0.016	0.000	0.460	Serine/threonine-protein kinase/endoribonuclease IRE1-like - Inside
136636_15	8	21,903,346	T	C	0.080	0.007	0.104	0.051	Sulfotransferase family cytosolic 1B member 1-like ~19.3 kb
285008_5	8	23,212,049	A	T	0.095	0.004	0.117	0.196	Septin-2B-like – Inside
8_31342192	8	31,342,192	A	C	0.140	0.002	0.060	0.426	Sodium/glucose cotransporter 4 – Inside
13580_33	9	12,460,482	G	A	0.093	0.007	0.039	0.079	Protein PRRC1-A-like ~40.7 kb
9_34396132	9	34,396,132	A	T	0.082	0.020	0.000	0.460	Nuclear receptor 2C2-associated protein – Inside
9_34714048	9	34,714,048	T	A	0.075	0.013	0.000	0.230	Cyclic AMP-responsive element-binding protein 3-like protein 3 – Inside
37532_4	10	85,175	A	G	0.094	0.008	0.039	0.153	SRSF protein kinase 3 ~44.3 kb
10_1731406	10	1,731,406	C	T	0.106	0.012	0.000	0.440	Low-quality protein: heme-binding protein 2-like – Inside
233444_3	10	15,677,896	G	A	0.093	0.007	0.013	0.065	Glutathione S-transferase sigma class protein – Inside
142010_20	10	18,474,986	C	T	0.091	0.009	0.130	0.134	Integrator complex subunit 13 – Inside
255888_35	10	19,786,344	G	T	0.102	0.003	0.065	0.236	RIB43A-like with coiled-coils protein 2 – Inside

(Continued)

TABLE 6 Continued

SNP_ID	Chr	Position	Ref	Alt	F_{ST}	*p*-value	MD	MAF	Inside/closest gene – distance to gene
84100_31	10	20,829,424	C	A	0.114	0.003	0.052	0.133	Mitosis inhibitor protein kinase wee1– Inside
10_23286715	10	23,286,715	G	A	0.127	0.005	0.000	0.430	Seryl-tRNA synthetase – Inside
234122_25	10	37,058,575	T	A	0.100	0.005	0.208	0.095	Tyrosine-protein phosphatase non-receptor type 13-like – Inside
32590_17	11	5,177,924	G	C	0.118	0.002	0.208	0.103	Deleted in lung and esophageal cancer protein 1 – Inside
215174_5	12	18,315,989	A	G	0.284	0.000	0.273	0.310	Cathepsin L-like ~21.8 kb
126933_19	*12*	28,518,329	A	C	0.139	0.001	0.065	0.081	Poly [ADP-ribose] polymerase ~116.3 kb
268319_32	13	8,706,747	C	G	0.126	0.002	0.208	0.246	Synaptojanin-1-like – Inside
13_24547378	13	24,547,378	C	T	0.133	0.006	0.000	0.320	3-Ketoacyl-CoA thiolase, mitochondrial – Inside
149069_19	14	4,017,350	T	A	0.122	0.002	0.143	0.076	Cathepsin L1 – Inside
189805_32	14	20,522,861	A	C	0.102	0.005	0.182	0.054	Guanine nucleotide-binding protein subunit beta ~48.9 kb
146461_31	14	24,563,619	G	A	0.115	0.002	0.117	0.486	Receptor protein-tyrosine kinase – Inside
245986_32	15	19,221,254	G	C	0.102	0.004	0.208	0.452	Contactin-5 – Inside
15_30613820	15	30,613,820	T	A	0.147	0.001	0.000	0.130	Golgin subfamily A member 2 - Inside
16_14629719	16	14,629,719	A	G	0.114	0.012	0.000	0.350	Importin-5 – Inside
45581_8	17	1,657,437	A	G	0.101	0.003	0.039	0.059	NRIF2 (Fragment) – Inside
294497_25	17	4,573,773	T	C	0.109	0.003	0.221	0.444	Acetylcholine receptor subunit alpha-type acr-7-like ~29.6 kb
17_11850848	17	11,850,848	T	A	0.269	0.002	0.300	0.243	DUF4149 domain-containing protein – Inside
263423_32	18	16,930,883	T	C	0.107	0.003	0.182	0.069	Fumarylacetoacetase-like – Inside
258399_24	18	17,697,950	T	C	0.108	0.005	0.260	0.153	IgGFc-binding protein-like – Inside
18_20951750	18	20,951,750	T	C	0.084	0.021	0.000	0.490	Cathepsin L - Inside
19_13393947	19	13,393,947	A	G	0.118	0.009	0.100	0.367	Nuclear receptor subfamily 4 group A member 2 – Inside
83241_35	19	19,121,785	C	T	0.097	0.006	0.026	0.100	Dynein heavy chain 7, axonemal-like – Inside

Chr, Chromosome; Position, SNP mapping (bp) within the assembled chromosome/scaffold of C. edule. MAF, Minimum allele frequency; MD, Missing data; F_{ST}, genetic differentiation; Ref, allelic variant in the genome; Alt, alternative variant.

the 110 outlier subset, and especially with the 156 exposure outliers dataset in the *Marteilia*-exposed sample (SLO19). Additionally, this sample showed significantly higher genetic diversity with outlier loci than non-exposed samples (0.169 *vs* 0.100; $p < 0.05$). We cannot rule out other types of selection operating on these markers/genes, i.e. diversifying selection, but currently there is not a straightforward explanation for these observations. New data coming from an ongoing common garden experiment carried out in Ría de Arousa with this set of markers should shed some light on the pattern of genetic diversity observed.

Low genetic structure was detected in Ría de Arousa using the whole SNP dataset, as previously reported for small geographic areas with microsatellites (Martínez et al., 2013) and SNPs (Coscia et al., 2020) in the common cockle, and in other mollusc species with microsatellites (Diz and Presa, 2009; Vera et al., 2016). This observation points towards the high dispersal capacity of the larvae while they remain in the water column. However, we identified 156 and 74 consistent outlier loci when comparing exposed and non-exposed samples and when considering the period of collection, respectively. Among them, a total of 110 outliers were close to DEG reported by Pardo et al. (2022) and were selected as the most consistent ones from this approach. These SNPs were able to discriminate between naive *vs* affected samples and even between exposed and non-exposed samples from the 2018/19 outbreak, although with less statistical support. On the other hand, we identified 123 SNPs linked to DEGs detected in the same marteiliosis outbreak by Pardo et al. (2022), many of them related to key immune functions, which showed significant genetic differentiation among samples with different levels of infection. We speculate that these SNPs could be associated with allelic variants responsible for the differential expression and consequently under selection to marteiliosis pressure. This approach has been followed to identify markers within DEGs associated with resistance to pathologies or other traits in aquaculture species (Robledo et al., 2017; Robledo et al., 2020; Moraleda et al., 2021).

Finally, taking advantage of the chromosome-level genome assembly, we selected a final panel of the most consistent 60 SNPs, to design a cost-effective molecular tool putatively useful for the selection of resistant strains and management of cockle beds for their recovery. Validation of the "in silico" genotyping information of this SNP set with a robust genotyping tool is

being undertaken for its application in an ongoing common garden experiment in the Ría de Arousa, involving cockle stocks from naive and marteiliosis-affected shellfish beds to confirm their usefulness for discriminating resilient and susceptible cockles. This could be eventually used for the appropriate management and recovery of both cockle production and natural bed ecosystems in Galicia, which holds the most important shellfishery of this species in Spain.

Data availability statement

The transcriptomic data presented in this study are deposited in the NCBI repository (https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA945848), accession number PRJNA945848. Genotype information from 2b-RADseq has been included as supplementary material in the text (Table S1).

Author contributions

PM performed the population genomics and transcriptomics analyses and selected the SNP panel in collaboration with AdC, VM and CR. FC carried out SNP calling and filtering from transcriptomics data. BA designed custom scripts and supervised the bioinformatic analysis. HM called and genotyped SNPs from 2b-RAD libraries. PB constructed 2b-RAD libraries. AsC and ID were involved in sampling in the field and histological evaluation of infection. VA and MP conceived the study, supervised the project and revised the manuscript. All authors collaborated in the manuscript and approved the final version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2023.1057206/full#supplementary-material

References

Allam, B., and Raftos, D. (2015). Immune responses to infectious diseases in bivalves. *J. Invertebr. Pathol.* 131, 121–136. doi: 10.1016/j.jip.2015.05.005

Andersen, T. J., Lanuru, M., van Bernem, C., Pejrup, M., and Riethmueller, R. (2010). Erodibility of a mixed mudflat dominated by microphytobenthos and *Cerastoderma edule*, East Frisian Wadden Sea, Germany. *Estuar. Coast. Shelf Sci.* 87, 197–206. doi: 10.1016/j.ecss.2009.10.014

Audemard, C., Le Roux, F., Barnaud, A., Collins, C., Sautour, B., Sauriau, P. G., et al. (2002). Needle in a haystack: Involvement of the copepod *Paracartia grani* in the life-cycle of the oyster pathogen *Marteilia refringens*. *Parasitology* 124, 315–323. doi: 10.1017/S0031182001001111

Beaumont, M. A., and Nichols, R. A. (1996). Evaluating loci for use in the genetic analysis of population structure. *Proc. R. Soc B Biol. Sci.* 263, 1619–1626. doi: 10.1098/rspb.1996.0237

Besnier, F., and Glover, K. A. (2013). ParallelStructure: A r package to distribute parallel runs of the population genetics program STRUCTURE on multi-core computers. *PloS One* 8, e70651. doi: 10.1371/journal.pone.0070651

Beukema, J. J., and Dekker, R. (2005). Decline of recruitment success in cockles and other bivalves in the Wadden Sea: Possible role of climate change, predation on postlarvae and fisheries. *Mar. Ecol. Ser.* 287, 149–167. doi: 10.3354/meps287149

Bos, O. G., Hendriks, I. E., Strasser, M., Dolmer, P., and Kamermans, P. (2006). Estimation of food limitation of bivalve larvae in coastal waters of north-western Europe. *J. Sea Res.* 55, 191–206. doi: 10.1016/j.seares.2005.10.006

Bower, S. M., McGladdery, S. E., and Price, I. M. (1994). Synopsis of infectious diseases and parasites of commercially exploited shellfish. *Annu. Rev. Fish Dis.* 4, 1–199. doi: 10.1016/0959-8030(94)90028-0

Bruzos, A. L., Santamarina, M., García-souto, D., Díaz, S., Rocha, S., Zamora, J., et al. (2022). The evolution of two transmissible leukaemias colonizing the coasts of Europe. *bioRixiv*. doi: 10.1101/2022.08.06.503021

Burdon, D., Callaway, R., Elliott, M., Smith, T., and Wither, A. (2014). Mass mortalities in bivalve populations: A review of the edible cockle *Cerastoderma edule* (L.). *Estuar. Coast. Shelf Sci.* 150 (partB), 271–280. doi: 10.1016/j.ecss.2014.04.011

Carballal, M. J., Iglesias, D., Abollo, E., Cao, A., García, L., Ramilo, A., et al. (2019). "Looking for hosts of *Marteilia cochillia* in the zooplankton," in *19th International Conference on Diseases of Fish and Shellfish Abstract Book, European Association of Fish Pathologists*. (Porto - Portugal). 502.

Carmichael, R. H., Walton, W., and Clark, H. (2012). Bivalve-enhanced nitrogen removal from coastal estuaries. *Can. J. Fish. Aquat. Sci.* 69, 1131–1149. doi: 10.1139/F2012-057

Carrasco, N., Green, T., and Itoh, N. (2015). *Marteilia* spp. parasites in bivalves: A revision of recent studies. *J. Invertebr. Pathol.* 131, 43–57. doi: 10.1016/j.jip.2015.07.016

Carrasco, N., Hine, P. M., Durfort, M., Andree, K. B., Malchus, N., Lacuesta, B., et al. (2013). *Marteilia cochillia* sp. nov., a new *Marteilia* species affecting the edible cockle *Cerastoderma edule* in European waters. *Aquaculture* 412–413, 223–230. doi: 10.1016/j.aquaculture.2013.07.027

Catchen, J., Hohenlohe, P. A., Bassham, S., Amores, A., and Cresko, W. A. (2013). Stacks: an analysis tool set for population genomics. *Mol. Ecol.* 22, 3124–3140. doi: 10.1111/mec.12354

Ciutat, A., Widdows, J., and Readman, J. W. (2006). Influence of cockle *Cerastoderma edule* bioturbation and tidal-current cycles on resuspension of sediment and polycyclic aromatic hydrocarbons. *Mar. Ecol. Prog. Ser.* 328, 51–64. doi: 10.3354/meps328051

Coscia, I., Wilmes, S. B., Ironside, J. E., Goward-Brown, A., O'Dea, E., Malham, S. K., et al. (2020). Fine-scale seascape genomics of an exploited marine species, the common cockle *Cerastoderma edule*, using a multimodelling approach. *Evol. Appl.* 13, 1854–1867. doi: 10.1111/eva.12932

Creek, G. A. (1960). The development of the lamellibranch *Cardium edule* l. *Proc. Zool. Soc London* 135, 243–260. doi: 10.1111/j.1469-7998.1960.tb05843.x

Darriba, S., Iglesias, D., and Carballal, M. J. (2020). *Marteilia cochillia* is released into seawater *via* cockle *Cerastoderma edule* faeces. *J. Invertebr. Pathol.* 172, 107364. doi: 10.1016/j.jip.2020.107364

Dégremont, L., Nourry, M., and Maurouard, E. (2015). Mass selection for survival and resistance to OsHV-1 infection in crassostrea gigas spat in field conditions: Response to selection after four generations. *Aquaculture* 446, 111–121. doi: 10.1016/j.aquaculture.2015.04.029

de la Ballina, N. R., Villalba, A., and Cao, A. (2018). Proteomic profile of the European flat oyster *Ostrea edulis* haemolymph in response to bonamiosis and identification of candidate proteins as markers of resistance. *Dis. Aquat. Organ.* 128, 127–145. doi: 10.3354/dao03220

Diz, A. P., and Presa, P. (2009). The genetic diversity pattern of *Mytilus galloprovincialis* in Galician rías (NW Iberian estuaries). *Aquaculture* 287, 278–285. doi: 10.1016/j.aquaculture.2008.10.029

Ducrotoy, J.-P., Rybarczyk, H., Souprayen, S., Bachelet, G., Beukema, J. J., Desprez, M., et al. (1991). "A comparison of the population dynamics of the cockle (Cerastoderma edule, l.) in north-Western Europe," In M. Elliot and J.-P. Ducrotoy Eds. *Estuaries and Coast: Spatial and Temporal Inrercomparisons*. (Olsen and Olsen, pp. 173–183.

Eriksson, B. K., Westra, J., Van Gerwen, I., Weerman, E., Vander Zee, E., Vander Heide, T., et al. (2017). Facilitation by ecosystem engineers enhances nutrient effects in an intertidal system. *Ecosphere* 8, e02051. doi: 10.1002/ecs2.2051

Evanno, G., Regnaut, S., and Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. *Mol. Ecol.* 14, 2611–2620. doi: 10.1111/j.1365-294X.2005.02553.x

Excoffier, L., and Lischer, H. E. L. (2010). Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and windows. *Mol. Ecol. Resour.* 10, 564–567. doi: 10.1111/j.1755-0998.2010.02847.x

Farhat, S., Tanguy, A., Pales, E., Guo, X., Boutet, I., Smolowitz, R., et al. (2020). Identification of variants associated with hard clam, *Mercenaria mercenaria*, resistance to quahog parasite unknown disease. *Genomics* 112, 4887–4896. doi: 10.1016/j.ygeno.2020.08.036

Ferns, P. N., Rostron, D. M., and Siman, H. Y. (2000). Effects of mechanical cockle harvesting on intertidal communities. *J. Appl. Ecol.* 37, 464–474. doi: 10.1046/j.1365-2664.2000.00509.x

Foll, M., and Gaggiotti, O. (2008). A genome-scan method to identify selected loci appropriate for both dominant and codominant markers: A Bayesian perspective. *Genetics* 993, 977–993. doi: 10.1534/genetics.108.092221

Food and Agriculture Organization of the United Nations [FAO] (2021) *Fisheries and aquaculture. global capture production quantity, (1950 - 2020)*. Available at: https://www.fao.org/fishery/statistics-query/en/aquaculture/aquaculture_value (Accessed August 2, 2022).

Ford, S. E., and Haskin, H. H. (1987). Infection and mortality patterns in strains of oysters *Crassostrea virginica* selected for resistance to the parasite *Haplosporidium nelsoni* (MSX). *J. Parasitol.* 73, 368–376. doi: 10.2307/3282092

Gutierrez, A. P., Symonds, J., King, N., Steiner, K., Bean, T. P., and Houston, R. D. (2020). Potential of genomic selection for improvement of resistance to ostreid herpesvirus in pacific oyster (*Crassostrea gigas*). *Anim. Genet.* 51, 249–257. doi: 10.1111/age.12909

Han, Y., Tang, Y., Sun, S., Kim, T., Ju, K., Ri, S., et al. (2021). Modulatory function of calmodulin on phagocytosis and potential regulation mechanisms in the blood clam *Tegillarca granosa*. *Dev. Comp. Immunol.* 116, 103910. doi: 10.1016/j.dci.2020.103910

Hayward, P. J., and Ryland, J. S. (1995). *Handbook of the marine fauna of north-west europe. 2nd ed.* Eds. P. J. Hayward and J. S. Ryland (Oxford: Oxford University Press). doi: 10.1093/acprof:oso/9780199549443.001.0001

Hollenbeck, C. M., and Johnston, I. A. (2018). Genomic tools and selective breeding in molluscs. *Front. Genet.* 9. doi: 10.3389/fgene.2018.00253

Iglesias, D., Villalba, A., Cao, A., and Carballal, M. (2019). "Is natural selection enhancing resistance against marteiliosis in cockles recruited in the inner side of the ria of arousa?," in *19th international conference on diseases of fish and shellfish, Oporto, Portugal* (Porto, Portugal: European Association of Fish Pathologists), 184. Available at: https://eafp.org/wp-content/uploads/2020/01/2019-porto-abstract-book.pdf.

Iglesias, D., Villalba, A., Mariño, C., No, E., and Carballal, M. J. (2023). *Long-Term Survey Discloses a Shift in the Dynamics Pattern of an Emerging Disease of Cockles Cerastoderma Edule, Marteiliosis, And Raises Hypothesis to Explain it*. doi: 10.2139/ssrn.4381126

Jombart, T. (2008). Adegenet: A r package for the multivariate analysis of genetic markers. *Bioinformatics* 24, 1403–1405. doi: 10.1093/bioinformatics/btn129

Keenan, K., Mcginnity, P., Cross, T. F., Crozier, W. W., and Prodöhl, P. A. (2013). DiveRsity: An r package for the estimation and exploration of population genetics parameters and their associated errors. *Methods Ecol. Evol.* 4, 782–788. doi: 10.1111/2041-210X.12067

Kekäläinen, J., Vallunen, J. A., Primmer, C. R., Rättyä, J., and Taskinen, J. (2009). Signals of major histocompatibility complex overdominance in a wild salmonid population. *Proc. R. Soc B Biol. Sci.* 276, 3133–3140. doi: 10.1098/rspb.2009.0727

Kopelman, N. M., Mayzel, J., Jakobsson, M., Rosenberg, N. A., and Mayrose, I. (2015). CLUMPAK: A program for identifying clustering modes and packaging population structure inferences across K. *Mol. Ecol. Resour.* 15, 1179–1191. doi: 10.1111/1755-0998.12387

Kube, P. D., Cunningham, M., Dominik, S., Parkinson, S., Henshall, J., Finn, B., et al. (2011). *Enhancement of the pacific oyster selective breeding program*. FRDC and SeafoodCRC Final Report Project No. 2006/227. (Hobart, TAS, Australia: CSIRO Marine and Atmospheric Research).

Langmead, B., Trapnell, C., Pop, M., and Salzberg, S. L. (2009). Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. *Genome Biol.* 10, R25. doi: 10.1186/gb-2009-10-3-r25

Lauckner, G. (1983). Diseases of Mollusca: Bivalvia. In Ed. O. Kinne *Diseases of Marine Animals* (Hamburgh: Biologische Anstalt Helgoland) II (ed. O. Kinne) 805–817.

Leprêtre, M., Faury, N., Segarra, A., Claverol, S., Degremont, L., Palos-ladeiro, M., et al. (2021). Comparative proteomics of ostreid herpesvirus 1 and pacific oyster interactions with two families exhibiting contrasted susceptibility to viral infection. *Front. Immunol.* 11. doi: 10.3389/fimmu.2020.621994

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., et al. (2009). The sequence alignment/map format and SAMtools. *Bioinformatics* 25, 2078–2079. doi: 10.1093/bioinformatics/btp352

Li, Y., and Liu, J. (2018). StructureSelector: A web-based software to select and visualize the optimal number of clusters using multiple methods. *Mol. Ecol. Resour.* 18, 176–177. doi: 10.1111/1755-0998.12719

Møhlenberg, F., and Kiørboe, T. (1983). Burrowing and avoidance behaviour in marine organisms exposed to pesticide-contaminated sediment. *Mar. pollut. Bull.* 14, 57–60. doi: 10.1016/0025-326X(83)90192-3

Maia, F., Barroso, C. M., and Gaspar, M. B. (2021). Biology of the common cockle *Cerastoderma edule* (Linnaeus 1758) in ria de aveiro (NW portugal): Implications for fisheries management. *J. Sea Res.* 171, 102024. doi: 10.1016/j.seares.2021.102024

Martínez, L., Méndez, J., Insua, A., Arias-Pérez, A., and Freire, R. (2013). Genetic diversity and population differentiation in the cockle *Cerastoderma edule* estimated by microsatellite markers. *Helgol. Mar. Res.* 67, 179–189. doi: 10.1007/s10152-012-0314-3

Mascaró, M., and Seed, R. (2000). Foraging behavior of *Carcinus maenas* (L.): Comparisons of size-selective predation on four species of bivalve prey. *J. Shellfish Res.* 19, 283–291. doi: 10.1007/s002270100677

Montaudouin, X., Arzul, I., Cao, A., Carballal, M. J., Chollet, B., Correia, S., et al. (2021). *Catalogue of parasites and diseases of the common cockle cerastoderma edule - COCKLES PROJECT* (Aveiro: UA Editora – Universidade de Aveiro). doi: 10.34624/9a9c-9j21

Moraleda, C. P., Robledo, D., Gutiérrez, A. P., Del-Pozo, J., Yáñez, J. M., and Houston, R. D. (2021). Investigating mechanisms underlying genetic resistance to

salmon rickettsial syndrome in Atlantic salmon using RNA sequencing. *BMC Genomics* 22, 156. doi: 10.1186/s12864-021-07443-2

Moreira Sanmartín, R., Roberts, S., and Figueras, A. (2016). "Molluscs," in *Genomics in aquaculture*. Eds. S. MacKenzie and S. Jentoft (London: Elsevier), 223–245. doi: 10.1016/B978-0-12-801418-9.00009-3

Nanut, M. P., Sabotič, J., Jewett, A., and Kos, J. (2014). Cysteine cathepsins as regulators of the cytotoxicity of NK and T cells. *Front. Immunol.* 5. doi: 10.3389/fimmu.2014.00616

Narum, S. R., and Hess, J. E. (2011). Comparison of F_{ST} outlier tests for SNP loci under selection. *Mol. Ecol. Resour.* 11, 184–194. doi: 10.1111/j.1755-0998.2011.02987.x

Navas, J. I., López-Sanmartín, M., Perez-Miguel, M., and Drake, P. (2018). "Detection of *Martelia cochillia* in *Cerastoderma edule* from Huelva coast (Andalusia Spain), Montpellier," in *AQUA 2018 - we r aquaculture*, 463.

Nell, J. A., Smith, I. R., and McPhee, C. C. (2000). The Sydney rock oyster *Saccostrea glomerata* (Gould 1850) breeding programme: progress and goals. *Aquac. Res.* 31, 45–49. doi: 10.1016/S0044-8486(03)00133-9

Neumeier, U., Lucas, C. H., and Collins, M. (2006). Erodibility and erosion patterns of mudflat sediments investigated using an annular flume. *Aquat. Ecol.* 40, 543–554. doi: 10.1007/s10452-004-0189-8

Niu, D., Jin, K., Wang, L., Feng, B., and Li, J. (2013). Molecular characterization and expression analysis of four cathepsin l genes in the razor clam, *Sinonovacula constricta*. *Fish Shellfish Immunol.* 35, 581–588. doi: 10.1016/j.fsi.2013.06.001

Parada, J. M., and Molares, J. (2008). Natural mortality of the cockle *Cerastoderma edule* (L.) from the Ría of Arousa (NW Spain) intertidal zone. *Rev. Biol. Mar. Oceanogr.* 43, 501–511. doi: 10.4067/S0718-19572008000300009

Pardo, B. G., Fernández, C., Pampín, M., Blanco, A., Iglesias, D., Cao, A., et al. (2022). Transcriptome characterization of the common cockle (*Cerastoderma edule*) after exposure to a *Marteilia cochillia* outbreak. *bioRixiv*. doi: 10.1101/2022.10.18.512677

Pembleton, L. W., Cogan, N. O. I., and Forster, J. W. (2013). StAMPP: An r package for calculation of genetic differentiation and structure of mixed-ploidy level populations. *Mol. Ecol. Resour.* 13, 946–952. doi: 10.1111/1755-0998.12129

Penn, D. J. (2002). The scent of genetic compatibility: Sexual selection and the major histocompatibility complex. *Ethology* 108, 1–21. doi: 10.1046/j.1439-0310.2002.00768.x

Ponsero, A., Dabouineau, L., and Allain, J. (2009). Modelling of common European cockle *Cerastoderma edule* fishing grounds aimed at sustainable management of traditional harvesting. *Fish. Sci.* 75, 839–850. doi: 10.1007/s12562-009-0110-4

Pradeu, T., and Du Pasquier, L. (2018). Immunological memory: What's in a name? *Immunol. Rev.* 283, 7–20. doi: 10.1111/imr.12652

Pritchard, J., Stephens, M., and Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics* 155, 945–959. doi: 10.1093/genetics/155.2.945

Proestou, D. A., Vinyard, B. T., Corbett, R. J., Piesz, J., Allen, S. K., Small, J. M., et al. (2016). Performance of selectively-bred lines of eastern oyster, crassostrea virginica, across eastern US estuaries. *Aquaculture* 464, 17–27. doi: 10.1016/j.aquaculture.2016.06.012

Puechmaille, S. J. (2016). The program structure does not reliably recover the correct population structure when sampling is uneven: Subsampling and new estimators alleviate the problem. *Mol. Ecol. Resour.* 16, 608–627. doi: 10.1111/1755-0998.12512

Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M. A. R., Bender, D., et al. (2007). PLINK: A tool set for whole-genome association and population-based linkage analyses. *Am. J. Hum. Genet.* 81, 559–575. doi: 10.1086/519795

Ragone Calvo, L. M., Calvo, G. W., and Burreson, E. M. (2003). Dual disease resistance in a selectively bred eastern oyster, *Crassostrea virginica*, strain tested in Chesapeake bay. *Aquaculture* 220, 69–87. doi: 10.1016/S0044-8486(02)00399-X

Rakotomalala, C., Grangeré, K., Ubertini, M., Forêt, M., and Orvain, F. (2015). Modelling the effect of *Cerastoderma edule* bioturbation on microphytobenthos resuspension towards the planktonic food web of estuarine ecosystem. *Ecol. Modell.* 316, 155–167. doi: 10.1016/j.ecolmodel.2015.08.010

Robledo, D., Hamilton, A., Gutiérrez, A. P., Bron, J. E., and Houston, R. D. (2020). Characterising the mechanisms underlying genetic resistance to amoebic gill disease in Atlantic salmon using RNA sequencing. *BMC Genomics* 21, 271. doi: 10.1186/s12864-020-6694-x

Robledo, D., Rubiolo, J. A., Cabaleiro, S., Martínez, P., and Bouza, C. (2017). Differential gene expression and SNP association between fast- and slow-growing turbot (*Scophthalmus maximus*). *Sci. Rep.* 7, 12105. doi: 10.1038/s41598-017-12459-4

Rochette, N. C., Rivera-Colón, A. G., and Catchen, J. M. (2019). Stacks 2: Analytical methods for paired-end sequencing improve RADseq-based population genomics. *Mol. Ecol.* 28, 4737–4754. doi: 10.1111/mec.15253

Ronza, P., Cao, A., Robledo, D., Gómez-Tato, A., Álvarez-Dios, J. A., Hasanuzzaman, A. F. M., et al. (2018). Long-term affected flat oyster (*Ostrea edulis*) haemocytes show differential gene expression profiles from naïve oysters in response to *Bonamia ostreae*. *Genomics* 110, 390–398. doi: 10.1016/j.ygeno.2018.04.002

Rousset, F. (2008). GENEPOP'007: a complete re-implementation of the GENEPOP software for windows and Linux. *Mol. Ecol. Resour.* 8, 103–106. doi: 10.1111/j.1471-8286.2007.01931.x

Rowley, A. F., Cross, M. E., Culloty, S. C., Lynch, S. A., Mackenzie, C. L., Morgan, E., et al. (2014). The potential impact of climate change on the infectious diseases of commercially important shellfish populations in the Irish Sea–a review. *ICES J. Mar. Sci.* 71, 741–759. doi: 10.1093/icesjms/fst234

Sambade, I. M., Casanova, A., Blanco, A., Gundappa, M. K., Bean, T. P., Macqueen, D. J., et al. (2022). A single genomic region involving a putative chromosome rearrangement in flat oyster (*Ostrea edulis*) is associated with differential host resilience to the parasite *Bonamia ostreae*. *Evol. Appl.* 00, 1–15. doi: 10.1111/eva.13446

Smits, M., Enez, F., Ferraresso, S., Dalla Rovere, G., Vetois, E., Auvray, J. F., et al. (2020). Potential for genetic improvement of resistance to *Perkinsus olseni* in the Manila clam, *Ruditapes philippinarum*, using DNA parentage assignment and mass spawning. *Front. Vet. Sci.* 7. doi: 10.3389/fvets.2020.579840

Stanley, R. R. E. E., Jeffery, N. W., Wringe, B. F., DiBacco, C., and Bradbury, I. R. (2017). GENEPOPEDIT: a simple and flexible tool for manipulating multilocus molecular data in r. *Mol. Ecol. Resour.* 17, 12–18. doi: 10.1111/1755-0998.12569

Subsecretaría Subdirección General de Análisis Coordinacion y Estadística (2020) *Estadísticas pesqueras noviembre 2020 [Fishery statistics November 2020]* (Ministerio de Agricultura Pesca y Alimentación). Available at: https://www.mapa.gob.es/es/estadistica/temas/estadisticas-pesqueras/ (Accessed June 15, 2022).

Sutherland, W. J. (1982). Do oystercatchers select the most profitable cockles? *Anim. Behav.* 30, 853. doi: 10.1016/S0003-3472(82)80159-0

Swanberg, I. (1991). The influence of the filter-feeding bivalve *Cerastoderma edule* l. on microphytobenthos: a laboratory study. *J. Exp. Mar. Bio. Ecol.* 151, 93–111. doi: 10.1016/0022-0981(91)90018-R

Tyler-Walters, H. (2007). "Cerastoderma edule (Common cockle)," in *Mar. life inf. netw. biol. sensit. key inf. rev*. Eds. H. Tyler-Walters and K. Hiscock (United Kingdom: Plymouth Mar. Biol. Assoc), 1–23. doi: 10.17031/marlinsp.1384.1

van der Schatte Olivier, A., Jones, L., Vay, L., Christie, M., Wilson, J., and Malham, S. K. (2020). A global review of the ecosystem services provided by bivalve aquaculture. *Rev. Aquac.* 12, 3–25. doi: 10.1111/raq.12301

Vera, M., Carlsson, J., Carlsson, J., Cross, T., Lynch, S., Kamermans, P., et al. (2016). Current genetic status, temporal stability and structure of the remnant wild European flat oyster populations: conservation and restoring implications. *Mar. Biol.* 163, 239. doi: 10.1007/s00227-016-3012-x

Vera, M., Maroso, F., Wilmes, S. B., Hermida, M., Blanco, A., Fernández, C., et al. (2022). Genomic survey of edible cockle (*Cerastoderma edule*) in the northeast Atlantic: a baseline for sustainable management of its wild resources. *Evol. Appl.* 15, 262–285. doi: 10.1111/eva.13340

Vera, M., Pardo, B. G., Cao, A., Vilas, R., Fernández, C., Blanco, A., et al. (2019). Signatures of selection for bonamiosis resistance in European flat oyster (*Ostrea edulis*): New genomic tools for breeding programs and management of natural resources. *Evol. Appl.* 12, 1781–1796. doi: 10.1111/eva.12832

Vigneron, N., and Van den Eynde, B. J. (2014). Proteasome subtypes and regulators in the processing of antigenic peptides presented by class I molecules of the major histocompatibility complex. *Biomolecules* 4, 994–1025. doi: 10.3390/biom4040994

Villalba, A., Iglesias, D., Ramilo, A., Darriba, S., Parada, J. M., No, E., et al. (2014). Cockle *Cerastoderma edule* fishery collapse in the Ria de Arousa (Galicia, NW Spain) associated with the protistan parasite marteilia cochillia. *Dis. Aquat. Organ.* 109, 55–80. doi: 10.3354/dao02723

Wang, S., Meyer, E., Mckay, J. K., and Matz, M. V. (2012). 2b-RAD: a simple and flexible method for genome-wide genotyping. *Nat. Methods* 9, 808–810. doi: 10.1038/nmeth.2023

Yao, T., Lu, J., Bai, C., Xie, Z., and Ye, L. (2021). The enhanced immune protection in small abalone *Haliotis diversicolor* against a secondary infection with. *Vibrio harveyi*. *Front. Immunol.* 12. doi: 10.3389/fimmu.2021.685896